

10600776

FILE 'CAPLUS' ENTERED AT 15:44:48 ON 05 APR 2005

L1 0 (DIPICOLIN? OR DPA) AND (FUJIWARA (2A) REACT?)

L2 370 (BACTERI? OR BACIL? OR MICROORGANISM? OR ANTHRAX) (S)  
(DIPICOLIN? OR DPA)

L3 3 L2 AND (CHCL3 OR CHLOROFORM)

L4 6 (BACTERI? OR BACIL? OR MICROORGANISM? OR ANTHRAX) AND  
FUJIWARA

L5 40 L2 AND (FLUORESC? OR ABSORBANCE OR PHOTOMETR? OR COLOR?)

L4 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:825177 CAPLUS

DOCUMENT NUMBER: 140:81782

TITLE: Evaluating trihalomethane content in drinking water on the basis of common  
monitoring parameters: regression models

AUTHOR(S): Espigares, Miguel; Lardelli, Pablo; Ortega, Pedro

CORPORATE SOURCE: Department of Preventive Medicine and Public Health,  
School of Pharmacy, University of Granada, Granada, E-18071, Spain

SOURCE: Journal of Environmental Health (2003), 66(3), 9-13

CODEN: JEVHAH; ISSN: 0022-0892

PUBLISHER: National Environmental Health Association

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The occurrence of trihalomethanes (THM) in drinking water sources is an issue of great interest due to their neg. impact on human health. This study correlated the THM occurrence with more routinely monitored water quality parameters to facilitate THM control. Water collected at various treatment stages were analyzed for the presence of THM using the Fujiwara method. Data collected from these detns. were compared with values obtained for free residual Cl<sub>2</sub> and combined residual Cl<sub>2</sub> concns. and with standard physicochem. and microbiol. indicators (COD, total chlorophyll, elec. conductivity, pH, alkalinity, turbidity, Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, NH<sub>3</sub>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, heterotrophic bacteria count, Pseudomonas species, total and fecal coliform and fecal streptococci bacteria). Data from these detns. were compiled and statistically analyzed to determine which variables correlated best with the presence and quantity of THM. THM concns. in water seemed to correlate directly with concns. of combined residual Cl<sub>2</sub> and NO<sub>3</sub><sup>-</sup>, and inversely with concns. of free residual Cl<sub>2</sub>. Multiple linear regression was performed to determine the best-fitting models. Models chosen incorporated from 2 to 4 independent variables and included COD, NO<sub>2</sub><sup>-</sup>, and NH<sub>3</sub>. These indicators, commonly determined during water treatment, demonstrated the strongest correlation with THM concns. in water and offered great utility as an accessible method for THM detection and control. REFERENCE COUNT: 20

L5 ANSWER 9 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:223060 CAPLUS

TITLE: Improving the performance of point-of-care and bio-warfare detection methods that use portable fluorescence spectrometers

AUTHOR(S): Hill, Ryan J.; DeRose, Paul C.

CORPORATE SOURCE: Department of Chemical Engineering, North Carolina State University, Raleigh, NC, 27695, USA

SOURCE: Abstracts of Papers, 227th ACS National Meeting, Anaheim, CA, United States, March 28-April 1, 2004 (2004), CHED-743. American Chemical Society: Washington, D. C.

CODEN: 69FGKM

DOCUMENT TYPE: Conference; Meeting Abstract

LANGUAGE: English

AB Fluorescence spectrometric methods are being used for detection of bio-warfare agents. These methods use portable fluorometers for in-field bio-agent detection by first responders. Fluorescent stds. need to be developed to calibrate these instruments and validate the corresponding assays. Here we target anthrax endospore derived dipicolinic acid (DPA) by complexing it with terbium in solution and quantifying the DPA concentration using fluorescence detection. A comparison of portable and benchtop fluorometer performance is done to determine fluorescence intensities as a function of DPA concentration and detection limits of DPA. DPA concns. are also correlated with corresponding anthrax endospore concns. Both types of fluorometers are calibrated using conventional and novel stds. and techniques. Results show a significant difference in the limit of detection between the research-grade fluorometer and the portable fluorometer. Ways to increase the sensitivity of portable fluorometers for detection of anthrax are proposed, including the development of suitable microfluidic devices.

L5 ANSWER 10 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:65964 CAPLUS

DOCUMENT NUMBER: 141:169072

TITLE: Interaction of dipicolinic acid with water-soluble and immobilized porphyrins

AUTHOR(S): White, Brandy J.; Legako, J. Andrew; Harmon, H. James

CORPORATE SOURCE: Department of Physics, Oklahoma State University, Stillwater, OK, 74078, USA

SOURCE: Sensors and Actuators, B: Chemical (2004), B97(2-3), 277-283

CODEN: SABCEB; ISSN: 0925-4005

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Dipicolinic acid (DPA), a constituent of bacterial spores, complexes with meso-tetra(4- sulfonatophenyl)porphine (TPPS), tetra(4-aminophenyl) porphyrin (NH<sub>2</sub>-TPP), monosulfonate tetra-Ph porphyrin (TPPS1), and meso-tetra(4- carboxyphenyl)porphine (CTPP4) resulting in new absorbance peaks in the visible spectra of the porphyrins at 419, 470, 441, and 415 nm, resp., with the intensity of these peaks showing linear dependence on DPA concentration. DPA can be detected at levels below 3 ppb by the porphyrin TPPS1 in aqueous solution. Detection of DPA using immobilized porphyrins is also demonstrated with detection limits of 16 ppb with NH<sub>2</sub>-TPP immobilized on glass, 14 ppb with NH<sub>2</sub>-TPP on cellulose fiber, and 1.5 ppb with TPPS1 on cellulose fiber.

REFERENCE COUNT: 28

L5 ANSWER 11 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:610737 CAPLUS

DOCUMENT NUMBER: 139:146159

TITLE: Methods and apparatus for assays of bacterial spores

INVENTOR(S): Ponce, Adrian; Bearman, Gregory H.

PATENT ASSIGNEE(S): California Institute of Technology, USA

SOURCE: PCT Int. Appl., 45 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

PATENT NO. KIND DATE APPLICATION NO. DATE

-----  
WO 2003065009 A2 20030807 WO 2003-US3036 20030131

WO 2003065009 A3 20040122

US 2004014154 A1 20040122 US 2003-355462 20030131

EP 1478912 A2 20041124 EP 2003-707656 20030131

PRIORITY APPLN. INFO.: US 2002-353268P P 20020201

US 2002-395372P P 20020712 US 2002-414170P P 20020927

WO 2003-US3036 W 20030131

AB A sample of unknown bacterial spores is added to a test strip. The sample of unknown bacterial spores is drawn to a first sample region on the test strip by capillary action. Species-specific antibodies are bound to the sample when the unknown bacterial spores match the species-specific antibodies, otherwise the sample is left unbound. DPA is released from the bacterial spores in the bound sample. The terbium ions are combined with the DPA to form a Tb-DPA complex. The combined terbium ions and DPA are excited to generate a luminescence characteristic of the combined terbium ions and DPA to detect the bacterial spores. A live/dead assay is performed by a release of the DPA for live spores and a release of DPA for all spores. The detection concns. are compared to determine the fraction of live spores. Lifetime-gated measurements of bacterial spores to eliminate any fluorescence background from organic chromophores comprise labeling the bacterial spore contents with a long-lifetime lumophore and detecting the luminescence after a waiting period. Unattended monitoring of bacterial spores in the air comprises the steps of collecting bacterial spores carried in the air and repeatedly performing the Tb-DPA detection steps above. The invention is also apparatus for performing the various methods disclosed above.

L5 ANSWER 14 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:585509 CAPLUS

DOCUMENT NUMBER: 139:97645

TITLE: "Real time viability detection of bacterial spores"

INVENTOR(S): *Vanderberg, Laura A.; Herdendorf, Timothy J.; Obiso, Richard J.*

PATENT ASSIGNEE(S): The Regents of the University of California, USA

SOURCE: U.S., 7 pp.

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

PATENT NO. KIND DATE APPLICATION NO. DATE

-----  
US 6599715 B1 20030729 US 2000-570137 20000512

PRIORITY APPLN. INFO.: US 1999-133823P P 19990512

AB This invention relates to a process for detecting the presence of viable bacterial spores in a sample and to a spore detection system, the process including placing a sample in a germination medium for a period of time sufficient for commitment of any present viable bacterial spores to occur, mixing the sample with a solution of a lanthanide capable of forming a fluorescent complex with dipicolinic acid, and, measuring the sample for the presence of dipicolinic acid, and the system including a germination chamber having inlets from a sample chamber, a germinant chamber and a bleach chamber, the germination chamber further including an outlet through a filtering means, the outlet connected to a detection chamber, the detection chamber having an inlet from a fluorescence promoting metal chamber and the detection chamber including a spectral excitation source and a means of measuring emission spectra from a sample, the detection chamber further connected to a waste chamber. A germination reaction mixture useful for promoting commitment of any viable bacterial spores in a sample including a combination of L-alanine, L-asparagine and D-glucose is also described.

REFERENCE COUNT: 11

L5 ANSWER 16 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:454934 CAPLUS

DOCUMENT NUMBER: 138:397532

TITLE: "Colorimetric method for detection of dipicolinic acid in mailpieces"

INVENTOR(S): *Robinson, William L.*

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 4 pp.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

PATENT NO. KIND DATE APPLICATION NO. DATE

-----  
US 2003108981 A1 20030612 US 2001-6150 20011210

PRIORITY APPLN. INFO.: US 2001-6150 20011210

AB Dipicolinic acid (pyridine 2,6 dicarboxylic acid) is a major component of bacterial spores and is unique in that it has only been found in spores. Dipicolinic acid is synthesized during sporulation and is released during germination or upon hydrolysis or heating. Current methods of anal. are based upon UV or spectroscopic absorption techniques. Though accurate these methods are time consuming and laborious. This invention describes a convenient colorimetric method that utilizes the color complex formed by the interaction of ferrous iron and reducing agents with dipicolinic acid. The presence of dipicolinic acid from spores or toxins produced by vegetative bacterial agents such as ammonia or other volatile amines within a package or container upon which the indicator is mounted will cause an immediate and slowly reversible color change.

Primary public use envelopes and personal protection badges or rings worn inside a room with potentially contaminated mail are specifically suggested.

L5 ANSWER 18 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:670526 CAPLUS

DOCUMENT NUMBER: 137:334779

TITLE: "FAST CARS: Engineering a laser spectroscopic technique for rapid identification of bacterial spores"

AUTHOR(S): *Scully, M. O.; Kattawar, G. W.; Lucht, R. P.; Opatrny, T.; Pilloff, H.; Rebane, A.; Sokolov, A. V.; Zubairy, M. S.*

CORPORATE SOURCE: Institute for Quantum Studies, Texas A and M University, College Station, TX, 77843, USA

SOURCE: **Proceedings of the National Academy of Sciences of the United States of America (2002), 99(17), 10994-11001**

CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Airborne contaminants, e.g., bacterial spores, are usually analyzed by time-consuming microscopic, chemical, and biol. assays. Current research into real-time laser spectroscopic detectors of such contaminants is based on e.g., resonance fluorescence. The present approach derives from recent expts. in which atoms and mols. are prepared by one (or more) coherent laser(s) and probed by another set of lasers. However, generating and using maximally coherent oscillation in macromols. having an enormous number of degrees of freedom is challenging. In particular, the short dephasing times and rapid internal conversion rates are major obstacles. However, adiabatic fast passage techniques and the ability to generate combs of phase-coherent femtosecond pulses provide tools for the generation and utilization of maximal quantum coherence in large mols. and biopolymers. We call this technique FAST CARS (femtosecond adaptive spectroscopic techniques for coherent anti-Stokes Raman spectroscopy), and the present article proposes and analyses ways in which it could be used to rapidly identify preselected mols. in real time. REFERENCE COUNT: 66

L5 ANSWER 21 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2000:740949 CAPLUS

DOCUMENT NUMBER: 134:233815

TITLE: Physical perturbation for fluorescent characterization of microorganism particles

AUTHOR: Bronk, Burt V.; Shoaibi, Azadeh; Nudelman, Raphael; Akinyemi, Agnes N.

CORPORATE SOURCE: AFRL/ECBC at U.S. Army ECBC, A.P.G., MD, 21010-5424, USA

SOURCE: Proceedings of SPIE-The International Society for Optical Engineering (2000), 4036(Chemical and Biological Sensing), 169-180

CODEN: PSISDG; ISSN: 0277-786X

PUBLISHER: SPIE-The International Society for Optical Engineering

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The motivation for using response to phys. perturbation to classify microparticles came from our previous expts. with Dipicolinic Acid (DPA). DPA as a calcium complex is a major component of bacterial spores, constituting more than 5% of their dry weight. It is not commonly found in other natural products and therefore its presence is indicative of the presence of bacterial spores. Previous schemes utilizing the presence of DPA to detect these spores have relied on fluorescence which occurs when lanthanide metals (e.g., terbium) are added to a solution where the presence of DPA is to be determined. We have recently demonstrated that changes in the fluorescence of DPA can be stimulated without the addition of such reagents. Thus after exposure to UV light, a substantial increase of fluorescence emitted by DPA solns. with a peak at 410 nm occurs for excitation light with wavelength less than approx. 305 nm.

REFERENCE COUNT: 11

L5 ANSWER 24 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1999:714833 CAPLUS

DOCUMENT NUMBER: 132:75561

TITLE: "Dipicolinic acid (DPA) assay revisited and appraised for spore detection"

AUTHOR(S): *Hindle, Alistair A.; Hall, Elizabeth A. H.*

CORPORATE SOURCE: Inst. Biotechnol., University of Cambridge, Cambridge, CB2 1QT, UK

SOURCE: **Analyst (Cambridge, United Kingdom) (1999), 124(11), 1599-1604**

CODEN: ANALAO; ISSN: 0003-2654

PUBLISHER: Royal Society of Chemistry

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Delayed gate fluorescence detection of dipicolinic acid (DPA), a universal and specific component of bacterial spores, has been appraised for use in a rapid anal. method for the detection of low concns. of bacterial spores. DPA was assayed by fluorimetric detection of its chelates with lanthanide metals. The influence of the choice and concentration of lanthanide and buffer ions on the fluorescence assay was studied as well as the effects of pH and temperature. The optimal system quantified the fluorescence of terbium monodipicolinate in a solution of  $10^{-10}$  M terbium chloride buffered with 1 M sodium acetate, pH 5.6 and had a detection limit of 2 nM DPA. This assay allowed the first real-time monitoring of the germination of bacterial spores by continuously quantifying exuded DPA. A detection limit of  $10^4$  *Bacillus subtilis* spores ml<sup>-1</sup> was reached, representing a substantial improvement over previous rapid tests.

REFERENCE COUNT: 51

L5 ANSWER 25 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1999:338095 CAPLUS

DOCUMENT NUMBER: 131:141627

TITLE: "Fluorescence of dipicolinic acid as a possible component of the observed UV emission spectra of bacterial spores"

AUTHOR(S): *Nudelman, Raphael; Feay, Nicole; Hirsch, Mathew; Efrima, Shlomo; Bronk, Burt*

CORPORATE SOURCE: Mantech Environmental Technology Inc., USA  
SOURCE: Proceedings of SPIE-The International Society for Optical Engineering (1999), 3533(Air Monitoring and Detection of Chemical and Biological Agents), 190-195  
CODEN: PSISDG; ISSN: 0277-786X  
PUBLISHER: SPIE-The International Society for Optical Engineering  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Dipicolinic acid (DPA) and the Ca<sup>2+</sup> complex of DPA (CaDPA) are well-known and are major chemical components of bacterial spores. DPA's native fluorescence is very weak and is thought to be completely masked by the fluorescence of tryptophan when this compound is present. Thus fluorescence related to DPA in spores is assumed by many authors to be completely absent. We show that the fluorescence of CaDPA is substantial for excitation between about 290 nm and 310 nm with emission peaking near 400 nm. This emission is at the long wavelength tail for emission from tryptophan. We examine whether the emission of CaDPA could contribute to the total emission spectrum when bacterial spores are present in an aerosol, for excitation wavelengths in the neighborhood of 310 nm. In this report we present measurements of fluorescence excitation and emission for CaDPA and compare them with that of DPA and tryptophan.  
REFERENCE COUNT: 15

L5 ANSWER 29 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 1990:403148 CAPLUS  
DOCUMENT NUMBER: 113:3148  
TITLE: "Chemical germination of native and cation-exchanged bacterial spores with trifluoperazine"  
AUTHOR(S): *Sacks, L. E.*  
CORPORATE SOURCE: West. Reg. Res. Cent., Agric. Res. Serv., Albany, CA, 94706, USA  
SOURCE: **Applied and Environmental Microbiology** (1990), **56**(4), 1185-7  
CODEN: AEMIDF; ISSN: 0099-2240  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB The calmodulin antagonist trifluoperazine and its analog chlorpromazine, both amphipaths, induced chemical germination of spores of various species, as do many surfactants. Cation load can greatly influence this response. Calmodulin antagonism does not seem to be involved. A new fluorometric assay for dipicolinic acid based on the fluorescence of the dipicolinic acid chelate of Tb<sup>3+</sup> was found to be simple and sensitive.

L5 ANSWER 31 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 1983:194476 CAPLUS  
DOCUMENT NUMBER: 98:194476  
TITLE: "Determination of dipicolinic acid in bacterial spores by derivative spectroscopy"  
AUTHOR(S): *Warth, Alan D.*  
CORPORATE SOURCE: Div. Food Res., CSIRO, North Ryde, 2113, Australia  
SOURCE: **Analytical Biochemistry** (1983), **130**(2), 502-5  
CODEN: ANBCA2; ISSN: 0003-2697

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Dipicolinic acid (DPA) was extracted from approx. 0.1 mg spores of 0.5 mL of sporulating culture with 20 mM HCl for 10 min at 100°. The suspension was diluted with 5 mM Ca<sup>2+</sup>, 100 mM Tris (pH 7.6), centrifuged, and the 1st derivative of the UV absorbance spectrum recorded from 275 to 285 nm. DPA concentration was determined from the difference between the maximum at 276.6 nm and the min. at 280 nm. The use of the difference between two 1st derivative values removed possible interference from sloping baselines. Turbidity, nucleic acid, and bacteriol. media did not interfere. Anal. time for 4 exts. was 4 min by using a spectrophotometer reading at 0.1-nm intervals. DPA at 0.1 mM gave 0.184 absorbance/nm at 25°. The relative standard deviation was 1.5%, and the detection limit 1 <SYM109>M.

L5 ANSWER 35 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1968:46869 CAPLUS

DOCUMENT NUMBER: 68:46869

TITLE: Modified reagent for dipicolinic acid analysis

AUTHOR(S): Rotman, Yigal; Fields, Marion L.

CORPORATE SOURCE: Univ. of Missouri, Columbia, MO, USA

SOURCE: Analytical Biochemistry (1968), 22(1), 168

CODEN: ANBCA2; ISSN: 0003-2697

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A modified Janssen reagent for colorimetric determination of dipicolinic acid in bacterial spores consists of 1% Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O and 0.1% cysteine in 0.05M pH 4.0 acetate buffer. The reagent is stable for 96 hrs. and produces colors identical in absorbance to those produced by a freshly prepared unmodified reagent.

L5 ANSWER 37 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1967:102395 CAPLUS

DOCUMENT NUMBER: 66:102395

TITLE: Determination of dipicolinic acid in bacterial spores by ultraviolet spectrometry of the calcium chelate

AUTHOR(S): Lewis, James Clement

CORPORATE SOURCE: Western Regional Res. Lab., Agr. Res. Serv., U.S. Dep. of Agr., Albany, CA, USA

SOURCE: Analytical Biochemistry (1967), 19(2), 327-37

CODEN: ANBCA2; ISSN: 0003-2697

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Dipicolinic acid is determined by spectrometry of the Ca chelate in 0.005M Ca(OH)<sub>2</sub>. Molar absorptivities at 25.0° are: 277.8 m<SYM109>, 4888 l./mole-cm.; 274.4, 3453; 269.8, 5362; 264.8, 3759; 263.3, 3814; 244, 1485. The equation:  $c = 33.8 (A_{277.8} - 2A_{274.4} + 2A_{269.8} - A_{264.8})$ , where c is the concentration of dipicolinic acid in <SYM109>g./ml. and A is the absorbance for 1 cm. cell-length at 25.0°, gives minimal interference by other components of exts. of bacterial spores. The temperature coefficient



for c is  $-0.22 \text{ g./ml./1}^\circ$ , or  $-0.64\%/1^\circ$ . Dipicolinic acid is extracted from spores with much less contamination by other uv absorbance by heating for 1 hr. at  $101^\circ$  in an a solution containing 71.8% EtOH and 1% HOAc than by heating in water.

L5 ANSWER 39 OF 40 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1958:35864 CAPLUS

DOCUMENT NUMBER: 52:35864

ORIGINAL REFERENCE NO.: 52:6478a-d

TITLE: Colorimetric assay for dipicolinic acid in bacterial spores

AUTHOR(S): Janssen, F. W.; Lund, A. J.; Anderson, L. E.

CORPORATE SOURCE: Univ. of Minnesota, Austin

SOURCE: Science (Washington, DC, United States) (1958), 127, 26-7

CODEN: SCIEAS; ISSN: 0036-8075

DOCUMENT TYPE: Journal

LANGUAGE: Unavailable

AB The complex (I) of ferrous iron and dipicolinic acid (2,6- pyridinedicarboxylic acid) was stabilized for 2 hrs. by adding ascorbic acid. Maximum color developed at pH 4-6. For assay, 5 ml. of a spore suspension was autoclaved for 15 min., cooled, acidified with 0.1 ml. of N AcOH, let stand 1 hr., and centrifuged. From the clear supernatant, 4 ml. was pipetted into a test tube, 1 ml. of fresh reagent (1% of  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)2.6\text{H}_2\text{O}$  and 1% of ascorbic acid in pH 5.5 acetate buffer) was added, and the color measured within 2 hrs. Reagent blank was negligible. The standard was 1 ml. of color reagent added to 4 ml. of aqueous solution containing 400  $\mu\text{g}$  of I. For comparison a spore suspension was extracted with ether, the extract was chromatographed, the spots containing I were eluted, and ultraviolet absorption measurements of the eluate gave 41.6  $\mu\text{g}$  of I. By the proposed assay, the same amount of the same suspension gave 42.3  $\mu\text{g}$  of I. Autoclaving of a spore suspension released 42.3  $\mu\text{g}$ /mg. of I compared with 43.0  $\mu\text{g}$ /mg. obtained by digesting the same suspension with 3N HCl for 15 min. Ferrous iron will complex with other  $\alpha$ -dicarboxyl pyridine compds., but only I has been found in spore preparation. Therefore, the proposed assay is specific for I in spore preparation.